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(54) Title: METHOD OF DIRECTING BIOSYNTHESIS OF SPECIFIC POLYKETIDES

(57) Abstract

A method to produce novel polyketide structures by designing and introducing specified changes in the DNA governing the synthesis of the polyketide is disclosed. The biosynthesis of specific polyketide analogs is accomplished by genetic manipulation of a polyketide-producing microorganism by isolating a polyketide biosynthetic gene-containing DNA sequence, identifying enzymatic activities associated within the DNA sequence, introducing one or more specified changes into the DNA sequence which codes for one of the enzymatic activities which results in an altered DNA sequence, introducing the altered DNA sequence into the polyketide-producing microorganism to replace the original sequence, growing a culture of the altered microorganism under conditions suitable for the formation of the specific polyketide analog, and isolating the specific polyketide analog from the culture. The method is most useful when the segment of the chromosome modified is involved in an enzymatic activity associated with polyketide biosynthesis, particularly for manipulating polyketide synthase genes from *Saccharapolyspora* or *Streptomyces*.

What is claimed is:

1. A method for directing the biosynthesis of specific polyketide analogs by genetic manipulation of a polyketide-producing microorganism, said method comprising the steps of:

(1) isolating a polyketide biosynthetic gene-containing DNA sequence;

(2) identifying enzymatic activities associated within said gene-containing DNA sequence;

(3) introducing one or more specified changes into said gene-containing DNA sequence which codes for one of said enzymatic activities resulting in an altered DNA sequence;

(4) introducing said altered DNA sequence into a polyketide-producing microorganism to replace the original sequence;

(5) growing a culture of the altered microorganism under conditions suitable for the formation of the specific polyketide analog; and

(6) isolating said specific polyketide analog from the culture.

2. The method of claim 1 wherein said polyketide biosynthetic gene-containing DNA sequence comprises genes which encode the enzymatic activities comprising a polyketide synthase.

3. The method of claim 2, wherein said polyketide synthase enzymatic activities comprise β-ketoreductase, dehydratase, acyl carrier protein, enoylreductase, β-ketoacyl ACP synthase, and acyltransferase.

4. The method of claim 1 wherein said alteration which occurs in the DNA sequence results in the inactivation of one or more enzymatic activities involved in the processing of the β-carbonyl of said polyketide.

5. The method of claim 4, wherein said inactivated enzymatic activities affecting the processing of the β-carbonyl of said polyketide comprise β-ketoreductase, dehydratase, and enoylreductase.

6. The method of claim 4 wherein said alteration in the DNA sequence results in the addition of one or more enzymatic activities involved in the β-carbonyl processing of said polyketide.

7. The method of claim 6 wherein said additional enzymatic activities are selected from the group consisting of β-ketoreductase, β-ketoreductase and dehydratase, and β-ketoreductase, dehydratase and enoylreductase.

8. The method of claim 1 wherein said alteration occurring in the DNA segment results in the inactivation of one or more enzymatic activities involved in the condensation of carbon units to the nascent polyketide structure.

9. The method of claim 8 wherein said enzymatic activities affecting the condensation of carbon units to the nascent polyketide structure comprise β-ketoacyl ACP synthase, acyl carrier protein and acyltransferase.

10. The method of claim 1 wherein said alteration in the DNA sequence results in the change of the length of the polyketide synthesized.

11. The method of claim 10 wherein said alteration results in the increase of the length of the polyketide.

12. The method of claim 11 wherein said alteration comprises the addition of DNA sequences encoding the enzymatic activities consisting of acyltransferase, acyl carrier protein and β-ketoacyl ACP synthase.

13. The method of claim 10 wherein said alteration results in the decrease of the length of the polyketide.

14. The method of claim 13 wherein said alteration consists of the deletion of a DNA segment between two sequences encoding corresponding enzymatic activities.

15. The method of claim 14 wherein said corresponding enzymatic activities are selected from the group consisting of β-ketoreductases, dehydratases, acyl carrier proteins, enoylreductases, β-ketoacyl ACP synthases, and acyltransferases.

16. The method of claim 1 wherein said alteration consists in the replacement of the DNA segment encoding an acyltransferase with a DNA segment encoding an acyltransferase of different specificity.

17. The method of claim 1 wherein said DNA sequence is isolated from a species from the *Actinomycetales* family.

18. The method of claim 17 wherein said DNA sequence is isolated from a genus selected from the group consisting of *Actinomyces, Dactylosporangium, Micromonospora, Nocardia, Sac., Streptoverticillium,* and *Streptomyces.*

19. The method of claim 17 wherein said genus is selected from the group consisting of *Saccharapolyspora* and *Streptomyces.*

20. The method of claim 19 wherein said genus is *Saccharapolyspora* and the species is *erythraea.*

21. The method of claim 19 wherein said genus is *Streptomyces* and the species is *hygroscopicus.*

22. The method of claim 1 wherein said polyketide is selected from the group consisting of macrolides, tetracyclines, polyethers, polyenes, ansaymcins and derivatives or analogs thereof.

23. The method of claim 22 wherein said polyketide is a macrolide.

24. The method of claim 23 wherein said macrolide is an erythromycin.

25. The method of claim 24 wherein said erythromycin analog is selected from the group consisting of 11-oxo-11-deoxyerythromycin A, 7-hydroxyerythromycin A, 6-deoxy-7-hydroxyerythromycin A, 7-oxoerythromycin A, 3-oxo-3-deoxy-5-desosaminylerythronolide A, Δ-6,7-. anhydroerythromycin A, ((14S,15S)14(1-hydroxyethyl)erythromycin A, 11-epifluoro-15-norerythromycinA, 14-(1-propyl)erythromycin A, 14(1-propyl)erythromycin A, and 14[1(1-hydroxypropyl)]erythromycin A.

26. The method of claim 1 wherein said DNA sequence, designated *eryA,* encodes the enzymatic activities associated with the formation of 6-deoxyerythronolide B.

27. The method of claim 26 wherein said DNA sequence comprises: the DNA sequence of Figure 2.

28. The method of claim 1 wherein said gene-containing DNA sequence encodes one or more enzymatic activities in the rapamycin biosynthetic pathway.

29. The method of claim 23 wherein said macrolide is a rapamycin analog.

30. A compound selected from the group consisting of 7-hydroxyerythromycin A; 6-deoxy-7-hydroxyerythromycin A; 7-oxoerythromycin A, 3-oxo-3-deoxy-5-desosaminyl-erythronolide A; Δ-6,7-anhydroerythromycin A; ((14S,15S)14(1-hydroxyethyl)erythromycin A; 11-epifluoro-15-norerythromycin A; 14-(1-propyl)erythromycin A; 14(1-propyl)erythromycin A; and 14[1(1-hydroxypropyl)]erythromycin A.